Allelic variants of hereditary prions: the bimodularity principle

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Abstract

Modern biology requires modern genetic concepts equally valid for all discovered mechanisms of inheritance, either “canonical” (mediated by DNA sequences) or epigenetic. Applying basic genetic terms such as “gene” and “allele” to protein hereditary factors is one of the necessary steps towards these concepts. The basic idea that different variants of the same prion protein can be considered as alleles has been previously proposed by Chernoff and Tuite. In this paper, the notion of prion allele is further developed. We propose the idea that any prion allele is a bimodular hereditary system that depends on a certain DNA sequence (DNA determinant) and a certain epigenetic mark (epigenetic determinant). Alteration of any of these two determinants may lead to establishment of a new prion allele. The bimodularity principle is valid not only for hereditary prions; it seems to be universal for any epigenetic hereditary factor.

Key words:

Prion, Amyloid, Prion Variant, Prion Strain, Conformational Template, The Bimodularity Principle, Epigenetic Inheritance

Text

Introduction

All fundamental genetic terms (gene, allele, genotype, mutation, recombination, etc.) were introduced just to describe genetic phenomenology and initially lacked any relation to certain types of biomolecules. After the genetic role of DNA had been demonstrated,[1,2] it has been strongly believed that all hereditary factors were represented by DNA, and that DNA sequencing was enough to unravel the origin of any hereditary differences in any species. As a result, all genetic terms became associated with specific processes affecting DNA sequences. Discovery of epigenetic, especially protein, inheritance opened a new era in biology and raised a lot of problems in genetic concepts. The fundamental genetic terms became fuzzy and thus called for reconsideration (for a review see refs. 3–5). Modern biology requires modern genetic concepts valid for all discovered mechanisms of inheritance, either "canonical" (mediated by DNA sequences) or epigenetic. One of the most intriguing epigenetic phenomena is protein inheritance, the field of genetics where hereditary factors are represented by proteins.

Currently, the scope of phenomena related to protein inheritance includes positive feedback by means of transcription factors,[6–9] cortical inheritance,[10] centriole inheritance,[11] and hereditary prions.[12,13] The latter are of special interest, because different variants (strains) of some hereditary prions have been disclosed (for a review see refs. 14–17). Each of these variants is a discrete hereditary factor and should be described in basic terms of general genetics; so, it is not surprising that prions are sometimes viewed as "protein genes".[18–20] Moreover, prionization, as well as prion curing, are considered as "protein mutations", and different variants (native and amyloid) of the same prion protein are called alleles.[21] In a recent issue of Seminars in Cell and Developmental Biology, different variants (native and amyloid) of the same prion protein were called alleles, and conversion of the [*prion*$^{-}$] state to the [*PRION*$^{+}$] state was designated as "protein paramutation".[22] The term "paramutation" is used when one allele is epigenetically converted after its presence in a heterozygote with another allele. Thus, the process of applying basic genetic terms for protein

hereditary factors has begun, and it is one of the key conditions necessary for the establishment of modern genetic concepts. Along the same lines of reasoning, we will consider different variants of the same hereditary prion as prion alleles. Current data concerning hereditary prion alleles are very complex and strongly need generalization. The aim of this paper is not to scrutinize the details, but to review the basic principles underlying hereditary prion alleles.

Molecular basics of hereditary prions

The term "prion" was introduced to designate a small proteinaceous infectious particle produced by the PrP protein in mammals.[23,24] Prion infectivity is based on prion self-perpetuation via changing the native protein isoform into the prion one, and newly appearing prion particles can be transmitted from one organism to another.[25] Taking into account the fact that in animals prions form only in somatic tissues, which do not transfer their properties to the descendants originating from the generative cells, prions had been considered as infectious agents only until 1994. Later, discovery of prions in some fungi substantially changed the initial paradigm: fungal prions are usually heritable as well.[26] In this review we will focus exactly on hereditary prions.

Currently, at least four molecular mechanisms underlying hereditary prion phenomenon are known: switch from native to amyloid conformation, positive feedback through protein phosphorylation by the MAPK-cascade, reproducible alterations in quaternary protein structure, and positive feedback through alterations in primary protein structure (Table 1). The first one has been extensively reviewed elsewhere (for a review see refs. 13,27–29), and therefore will be mentioned here just briefly.

Switch from native to amyloid conformation. The term "amyloids" means non-covalent protein aggregates that (i) form unbranched fibrils, (ii) possess cross-β-structures, and (iii) have a core region extremely resistant to hydrogen/deuterium exchange, proteases, and chemical denaturation.[30] All the above mentioned amyloid properties have been proven for several hereditary prions, at least

in vitro (Table 1), [31–38] while for some other prions these properties have been presumed rather than proven (for a review see ref. 13).

Amyloid prion aggregates are self-perpetuating because they induce conformational switch of a certain protein from its native to amyloid isoform, thus templating their own reproduction. Different amyloid templates can be produced on the base of the same native isoform (for a review see ref. 13). This phenomenon is in good agreement with the fact that the same amyloid prion exists in multiple alleles differing from each other in their manifestation.[14–17]

Heredity of amyloid prions is based on three processes (Fig. 1). The first is prion reproduction: a monomeric native protein interacts with a preexisting aggregate, changes its own conformation and incorporates into the amyloid fibril. As a result, the fibril elongates. The second process is prion multiplication:[39] the growing fibril is cleaved into fragments, producing aggregate seeds called propagons.[40] In most cases, cellular chaperone machinery performs this function; for instance, in the *Saccharomyces* yeast, the major role belongs to Hsp104 (for a review see ref. 13). The third process is prion inheritance. It is based on prion seed transmission from a cell to its progeny during cell division, mating, or hyphae conjugation.

Infectivity of fungal amyloid prions is typically provided by propagon transmission through cytoduction or local anastomoses. Moreover, it can be modeled using protein transformation with *in vitro* obtained prion aggregates or cellular lysates from a [*PRION*$^{+}$] strain.[41–43] Recently, it has been also proposed that propagons can be transmitted from cell to cell by extracellular vesicles.[44]

The most extensively studied amyloid hereditary prion is [*PSI*$^{+}$], an aggregated form of Sup35p in *Saccharomyces cerevisiae*.[14] In its native conformation, this protein is soluble and functions as a component of the translational termination machinery.[45,46] Under some rare events with frequency about 10^{-7} per cell,[47] it switches to atypically stable conformation, and the altered molecules are incorporated in the amyloid (for a review see ref. 39). When such aggregate interacts with the native Sup35 molecules, it converts them to the prion isoform too, and thus reproduces. Multiplication of this prion depends on Hsp104, which cleaves aggregates.[39]

[*PSI*$^{+}$] is effectively transmissible through cytoduction, and is stably heritable both mitotically and meiotically. It decreases the efficiency of translation termination and behaves as a non-Mendelian nonsense suppressor.[48] Multiple [*PSI*$^{+}$] alleles distinct in their suppressor efficiency, mitotic and meiotic stability, the proportion of aggregated Sup35p, the number of aggregates per cell, and some other features are described (for a review see refs. 14,15,17,40,49,50). Formation, heritability and elimination of [*PSI*$^{+}$] depend on various genetic and environmental factors thoroughly discussed in prion literature (for a review see refs. 13,27–29).

About a dozen of other amyloid hereditary prions are known in yeasts and *Podospora anserina*. The amyloid domains in these prions are non-homologous, and even distinct in their physical characteristics: some of them are N/Q rich, but others are not (for a review see refs. 12,13,18), so, the details of amyloid prionization seem to be specific in each case. Amyloid prions have been also described in mammals (for a review see ref. 51), but here they are only infectious, not heritable.

Positive feedback through protein phosphorylation by the MAPK-cascade. This mechanism has been described in the filamentous fungus *Podospora anserina*.[52,53] The MAPK-cascade is a regulatory phosphorylation system typical for all eukaryotes and comprising three sequentially functioning protein kinases: MAPKKK, MAPKK and MAPK, where MAPK is phosphorylated by MAPKK which in turn is a target for MAPKKK (for a review see ref. 54). *P. anserina* possesses three autonomous MAPK pathways: PaMpk1, PaMpk2 and PaMpk3.[55] Activation of the PaMpk1 pathway results in crippled growth, *i.e.* in formation of poorly growing female-sterile pigmented flat mycelium. This phenotype is infectious, and its molecular basic is designated as the *C* prion.[52] PaMpk1 is normally activated during stationary phase and ceases after return to growth; such activation is infectious through local anastomoses for normal recipient strains, but is not heritable within the initial mycelium. However, when this pathway is occasionally triggered during the growth phase, it undergoes self-activation (molecules in the ON state activate those in the OFF state) and appears to be both infectious and mitotically heritable (Figure 2A).[53] So, at least two

different forms of *C* are currently known: one is both infectious and mitotically heritable, and the other is only infectious.[53]

It is unclear which element of the PaMpk1 pathway directly corresponds to *C*. *C* manifestation requires all three genes of the pathway (*PaASK1*, *PaMKK1*, and *PaMPK1*) and can be induced in the normally growing mycelium when any of them is overexpressed.[53,55,56] So, it is possible that *C* is determined by the state of the PaMpk1 pathway as a whole, and not by the state of a certain protein kinase.[55]

C inheritance in the growing mycelium requires not only the PaMpk1 pathway, but also increased translational accuracy and some genes encoding NADPH oxidases.[53,55] Moreover, *C* can be cured by various stresses, including heat, UV light, some antibiotics, and high concentrations of sucrose.[52] The exact mechanisms of these effects are still obscure, but the appearance and the dissipation of *C* are undoubtedly under complex genetic, developmental and environmental control. The difference between *C* produced during the growing and the stationary phases is of special interest: the first is both infectious and heritable, while the second is only infectious.

Up to date, *C* remains the only known example of heritable unit caused by post-translational protein modification. However, since protein-based inheritance has been discovered just recently, other examples are possible. Theoretically, they can be determined by various types of protein modification, not only phosphorylation.

Reproducible alterations in quaternary protein structure. The unique example of this mechanism known so far has been described in the *Saccharomyces* yeast. It involves the complex of two non-homologous proteins: Pma1, an essential highly abundant P-type ATPase, and Std1, a component of the Snf3/Rgt2 regulatory pathway. Normally, Pma1 is associated mostly with the Std1 paralog Mth1. When Pma1 preferable interaction occasionally shifts from Mth1 to Std1, an abnormal protein complex designated as prion [GAR^+] forms and reproduces (Figure 2B).[57] Recently, it has been shown that [GAR^+] is also induced by the presence of unknown bacterial chemical factors.[58]

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In the [*GAR*$^+$] cells, glucose repression is modified: these cells can grow in glycerol in presence of non-metabolizable glucose analog, glucosamine.[57] This phenotype is transmissible via cytoduction, and is steadily inherited both mitotically and meiotically. [*GAR*$^+$] formation is enhanced under *STD1* or *PMA1* overexpression, while *MTH1* overexpression leads to the opposite effect. After [*GAR*$^+$] is established, it can be reversibly cured by transient lack of Hsp70 proteins Ssa1 and Ssa2. Moreover, [*GAR*$^+$] is totally cured when both *STD1* and the N-terminus of *PMA1* are deleted, but it reproduces in case only one of them is absent.[57] Thus, the exact molecular mechanism of [*GAR*$^+$] manifestation is unknown to date.

Positive feedback through alterations in primary protein structure. In all above mechanisms, the differences between the native and prion states do not affect primary protein structure. [*β*$^+$], a self-activating form of yeast protease B, is the unique example of the opposite situation.[59] Protease B (PrB) is derived from a large catalytically inactive zymogene encoded by the *PRB1* gene and undergoes several steps of maturation.[60] At final steps, the zymogene is truncated by protease A (PrA) and then by PrB itself: the mature molecules truncate the immature ones, thus producing a positive feedback loop.[61]

The effectiveness of this loop depends on several genetic and environmental factors. On YPAD medium, PrB self-activation is PrA-dependent.[62] As a result, deletion of *PEP4* (the gene coding for PrA) leads to gradual decrease and eventual loss of active PrB; however, this loss is delayed, and the residual PrB activity lasts at least for 20 mitotic divisions. This effect is called “phenotypic lag”.[63] On YPG medium, PrB is autonomous and does not require PrA activity; so, the cells display steady PrB self-activation even when *PEP4* is deleted. When such cells are transferred to YPAD, they eventually lose active PrB and fail to restore it after return to YPG (strictly speaking, the restoration is possible, but it requires *PRB1* overexpression). Thus, when *PRB1* is normally expressed, two kinds of cells having exactly the same DNA background and differing only in their PrB state can be obtained on YPG medium: PrB positive ([*β*$^+$]) and PrB negative ([*β*$^-$]).[59]

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[β^+] is stably heritable in both mitotic and meiotic generations, and can be effectively transmitted by cytoduction. So far, it is the unique hereditary factor reproducing through protein primary structure changes.

The bimodularity principle of hereditary prion alleles

According to conventional criteria, hereditary prions are (i) non-Mendelian elements; (ii) reversibly curable by anti-prion agents, (iii) depending on the corresponding gene, and (iv) capable to appear *de novo* when this gene is overexpressed.[26] The third point is of special importance for us. It means that allelic hereditary prions obligatorily depend on the same gene, and here is the way to uncover their allelism. For example, no [*URE3*] allele can be reproduced under the lack of the *URE2* gene, and hereby any of them is quickly lost.[26] So, all hereditary prions which are irreproducible in this DNA background should be considered as [*URE3*] alleles.

Without taking the fourth criterion into account, dependence on the same gene does not guarantee prion allelism yet: non-allelic hereditary prions may require the same molecular function for their multiplication, as in case of [*PSI*$^+$], [*PIN*$^+$] and [*URE3*], which are lost under *HSP104* deletion (for a review see ref. 13). Therefore, to prove that certain hereditary prions are allelic to each other, both the third and the fourth criteria should be met. This approach is successful even for those prion alleles which significantly differ in their manifestation, like strong [*PSI*$^+$] and [*ETA*$^+$].[49]

It is obvious from the above criteria that to perpetuate a certain [*PRION*$^+$] allele, two kinds of molecular structures are required: (i) the protein structure (chemically modified, truncated or conformationally altered) as a seed, and (ii) the corresponding DNA sequence, otherwise the prion will not be reproduced due to the lack of the necessary protein. So, a [*PRION*$^+$] allele is a bimodular hereditary system that depends on the certain DNA sequence (DNA determinant) and the certain epigenetic mark (epigenetic determinant). The first encodes the prion protein sequence, while the second describes the state of this material, and both affect prion functions and evolution.[64] Notably, the presence of a certain [*PRION*$^+$] allele in a cell does not mean that all molecules of the

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corresponding protein are transformed into the prion state: some portion of the native protein is also retained.[65–67] So, the symbol [*PRION*$^{+}$] signifies the availability of specific epigenetic mark, which is absent in the [*prion*$^{-}$] cells.

One can distinguish three types of differences between prion alleles. In the simplest case, these differences are solely of epigenetic origin, like between strong and weak [*PSI*$^{+}$] variants independently produced in the same *SUP35* background.[14] Such prion alleles are encoded by the identical DNA determinant and vary just in epigenetic marks. On the contrary, some prion alleles are identical in their epigenetic mark, but differ in the DNA determinant. This is typical to cytoductants with various *SUP35* backgrounds to which the same [*PSI*$^{+}$] template has been transmitted.[20,68] And finally, in most complicated cases, the differences between prion alleles affect both DNA and epigenetic determinants. The two distinct [*PSI*$^{+}$] prion variants are remarkable example: the strong one produced by the normal *SUP35* molecules, and the weak one induced in the *SUP35*^{PNM2} background (hereafter referred as strong [*PSI*$^{+}$] and [VH-1], respectively).[14,15] The fact that even such prion variants are allelic to each other can be proven by the following simple logic. [*VH-1*] is reproducible in the normal *SUP35* background.[15] This leads to the appearance of a new prion variant with altered DNA determinant but the same epigenetic mark. The new prion variant (it will be designated here as [*VH-1*]^{new}) is allelic to [*VH-1*] since they differ in the DNA determinant only. Meanwhile, [*VH-1*]^{new} is allelic to strong [*PSI*$^{+}$]: both are encoded by the same DNA determinant and differ just in epigenetic marks. As a result, [*VH-1*] is allelic to [*VH-1*]^{new}, and [*VH-1*]^{new} is, in turn, allelic to strong [*PSI*$^{+}$]; this means that [*VH-1*] and strong [*PSI*$^{+}$] are allelic as well.

Thus, prion alleles are considerably more sophisticated hereditary factors compared to DNA alleles or epialleles. Prion alleles are bimodular: their diversity displays variation in both DNA and epigenetic determinants, and alteration in either of these determinants can result in the appearance of a new prion allele. So, we propose bimodular designation of each prion allele: “DNA determinant [epigenetic determinant]”. It should be especially noted that the DNA determinant is not a part of

prion allele; its presence in a certain bimodular designation just definitely describes the corresponding protein sequence.

Usually both determinants of a certain prion are represented by a set of multiple variants, and each combination corresponds to a potential prion allele. This diversity is restricted by cell lethality or prion loss in specific combinations (see below). Some prion alleles are distinct in their manifestation, while some are phenotypically indistinguishable from each other, like DNA sequences with synonymous polymorphism.

Implications of the bimodularity principle for the [*PSI*$^+$] prion

As noted above, [*PSI*$^+$] exists in multiple alleles distinct in their mitotic and meiotic stability, nonsense-suppressor efficiency, the proportion of aggregated Sup35p, the number of propagons per cell, and some other properties. In addition, the absence of the prion particles is considered as a null-allele, [*psi*$^-$]. The aim of the following sections is to overview the principal variety of prion alleles and potential types of their interactions on the example of the [*PSI*$^+$] prion.

Prion alleles corresponding to the [*psi*$^-$] state. We propose to distinguish three classes of [*psi*$^-$] alleles. The first one corresponds to the reference *SUP35* sequence peculiar to laboratory strains (*SUP35*^{ref}; including known natural polymorphism; for a review see ref. 20) and native Sup35p; in this case, a cell possesses the appropriate DNA determinant but lacks the conformational template (*SUP35*^{ref}[*psi*$^-$]). If such null-allele is supplemented with aggregated Sup35p of a normal protein sequence (*SUP35*^{ref}[*PSI*$^+$]), it undergoes epigenetic conversion to the [*PSI*$^+$] state. Depending on which conformational template is transmitted (strong or weak, [*PSI*$^+$]^S or [*PSI*$^+$]^W, respectively), the initial null-allele can be converted to different *SUP35*^{ref}[*PSI*$^+$] alleles (for a review see ref. 69). Various [*PSI*$^+$] templates with altered protein sequence are also reproducible in the *SUP35*^{ref} background and provide epigenetic conversion of *SUP35*^{ref}[*psi*$^-$] as well.[15,20] At least one exception is currently known: *SUP35*^{ref} fails to reproduce [*PSI*$^+$] with the double substitution Q89K,Q90K.[70] Thus, *SUP35*^{ref}[*psi*$^-$] is convertible to the [*PSI*$^+$] state by many but not all conformational templates.

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Another class of [*psi*$^-$] alleles lacks both the conformational template and the DNA determinant. To be clear, *SUP35* is essential and therefore cannot be deleted as a whole; however, the N-terminal region of Sup35p does not affect viability but is required for the Sup35p prionization.[71,72] So, the N-truncated *SUP35* (*SUP35*$^{\Delta N}$) is insufficient for [*PSI*$^+$] formation and will be further referred as the DNA N-determinant absence. When *SUP35*$^{\Delta N}$[*psi*$^-$] is supplemented with any [*PSI*$^+$] through cytoduction or protein transformation, the transmitted prion particles do not receive the material for growth and therefore are not reproduced[69] ([*PSI*$^+$]$_{R-}$). As a result, the *SUP35*$^{\Delta N}$[*psi*$^-$] alleles are *per se* epigenetically inconvertible.

The difference between the *SUP35*^{ref}[*psi*$^-$] and the *SUP35*$^{\Delta N}$[*psi*$^-$] alleles is also evident by their ability to revert to the [*PSI*$^+$] state. *SUP35*^{ref}[*psi*$^-$] undergoes rare spontaneous reversions to *SUP35*^{ref}[*PSI*$^+$], and these events are strongly enhanced under the DNA N-determinant overexpression.[14] The reversion mechanism is still obscure; it admittedly relates to stochastic shifts from the native Sup35p conformation to the amyloid one, and another amyloid prion, [*PIN*$^+$], is required as an initial template for Sup35p aggregation.[73] Usually, various *SUP35*^{ref}[*PSI*$^+$] alleles can arise on the same *SUP35*^{ref}[*psi*$^-$] background;[14,15,17] so, the shift to the amyloid conformation may occur in several alternative ways, with some distinctions in the eventual folding (for a review see ref. 13). On the contrary, *SUP35*$^{\Delta N}$[*psi*$^-$] is completely irreversible because of the lack of the DNA N-determinant.

In the third, intermediate, class of [*psi*$^-$] alleles, the DNA N-determinant is present, but its sequence is altered compared to *SUP35*^{ref} due to point mutations or local deletions (*SUP35*^{alt}). To the best of our knowledge, all *SUP35*^{alt}[*psi*$^-$] alleles published so far are reversible. Moreover, they are epigenetically convertible to the [*PSI*$^+$] state, but specific conformational templates are usually required. One of the most famous examples is *SUP35*^{PNM2}[*psi*$^-$]: although it is able to form and perpetuate several specific conformational templates, it leads to loss of some *SUP35*^{ref}[*PSI*$^+$].[15,74,75] Similar features are characteristic to [*psi*$^-$] alleles with *sup35-M1* (Y46K/Q47K) or *sup35-M2*

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(Q61K/Q62K) affecting the first and the second oligonucleotide repeats in the DNA N-determinant respectively.[70]

Inability of a certain *SUP35*^{alt}[*psi*$^{-}$] allele to undergo epigenetic conversion by particular [*PSI*$^{+}$] templates may also be due to lethality of these combinations. For example, *sup35-2* is lethal with atypical [*PSI*$^{+}$] initially called [*ETA*$^{+}$][76] but is compatible with *SUP35*^{ref}[*PSI*$^{+}$]^{S}.[49] The point T341D mutation which affects the C-terminal region of Sup35p causes lethality with *SUP35*^{ref}[*PSI*$^{+}$]; however, the lethal effect is ceased when the DNA N-determinant is absent or unable to provide prionization.[77] Thus, the features of the prion alleles are conditioned by both N- and C-terminal regions of Sup35p. In theory, some completely irreversible and inconvertible *SUP35*^{alt}[*psi*$^{-}$] alleles may exist, but none has been discovered so far.

Prion alleles corresponding to the [*PSI*$^{+}$] state. A certain [*PSI*$^{+}$] allele is the bimodular system where native Sup35p molecules involved in [*PSI*$^{+}$] reproduction are encoded by a certain DNA determinant. So, by indicating this determinant for a [*PSI*$^{+}$] allele, we give definite description of the prion protein sequence. For example, *SUP35*^{ref}[*PSI*$^{+}$] designates a [*PSI*$^{+}$] allele in which prion particles are produced by the Sup35p molecules with reference protein sequence. Since different conformational templates can be derived from the same DNA determinant,[14,15,17] additional specifying notes, like *SUP35*^{ref}[*PSI*$^{+}$]^{S} or *SUP35*^{ref}[*PSI*$^{+}$]^{W}, are required. Also, in some *SUP35* backgrounds encoding only the N-domain of Sup35p (for example, *SUP35*$^{1\text{-}123}$ with additional *SUP35*$^{\Delta N}$ to provide viability), all [*PSI*$^{+}$] templates become undifferentiated, [*PSI*$^{+}$]^{U}.[69]

In most well-studied [*PSI*$^{+}$] alleles, the DNA determinant is *SUP35*^{ref}. Such alleles may significantly differ in their properties;[14,15,17,49] all distinctions are conditioned here by conformational templates specificity.[41–43] [*PSI*$^{+}$] alleles with the *SUP35*^{alt} DNA determinant are also known, and they are strongly variable depending on both determinants.[20]

Interaction between different [*psi*$^{-}$] alleles. If a diploid cell has got two different [*psi*$^{-}$] alleles from the parent strains (similar situation can be modeled in a [*psi*$^{-}$] haploid carrying two distinct copies of *SUP35*), these null-alleles should interact with each other with respect to their

reversibility to the [*PSI*$^{+}$] state. Depending on the combined null-alleles, the results of interaction may be diverse. We will focus just on several examples.

In the simplest cases, clear dominance is expected. For instance, when one null-allele is reversible and another is irreversible (*SUP35*$^{\Delta N}$[*psi*$^{-}$]), the first should dominate over the second. However, this effect cannot be detected in common way through arising of colonies with [*PSI*$^{+}$]-mediated nonsense suppression, since N-truncated Sup35p is never included in the prion particles and thus provides adequate termination at nonsense codons.[69]

Dominance may also take place when each null-allele is reversible alone, but one of them has PNM ("psi-no-more") manifestation. Indeed, if this effect of a certain *SUP35*^{PNM} encompasses all possible *SUP35*^{ref}[*PSI*$^{+}$] templates, the reversions occurred in the *SUP35*^{ref}[*psi*$^{-}$]/*SUP35*^{PNM}[*psi*$^{-}$] heterozygote should arise via *PNM*-compatible templates only, and *SUP35*^{PNM}[*psi*$^{-}$] will dominate over *SUP35*^{ref}[*psi*$^{-}$]. But when no PNM mutation is involved, each reversible null-allele can participate in the [*PSI*$^{+}$] state production, thus displaying some kind of co-dominant manifestation.

Two different [*psi*$^{-}$] alleles may also interact with each other in their convertibility to the [*PSI*$^{+}$] state, but here the transmitted conformational template is required. So, this phenomenon is *per se* very close to interaction between [*PSI*$^{+}$] and [*psi*$^{-}$] alleles, and will be considered in the following section.

Interaction between [*PSI*$^{+}$] and [*psi*$^{-}$] alleles. The simplest model to study such interaction is diploids produced by *SUP35*^{ref}[*PSI*$^{+}$] x *SUP35*^{ref}[*psi*$^{-}$] crosses. In these diploids, [*PSI*$^{+}$] dominates in all aspects of its manifestation.[50,78] Moreover, epiheterozygosity for [*PSI*$^{+}$] brings to protein paramutation: [*psi*$^{-}$] undergoes epigenetic conversion to [*PSI*$^{+}$], and tetrad analysis typically gives non-Mendelian 4[*PSI*$^{+}$] : 0[*psi*$^{-}$] segregation (for a review see ref. 22).

If two prion alleles combined in a [*PSI*$^{+}$] x [*psi*$^{-}$] hybrid considerably differ in their DNA determinants, interaction between them may be more complex. For example, in the *SUP35*^{ref}[*PSI*$^{+}$]/*SUP35*$^{\Delta N}$[*psi*$^{-}$] heterozygote, the first prion allele is dominant for [*PSI*$^{+}$] reproduction and recessive for nonsense-suppression (see above). All ascospores produced by such

heterozygotes receive *SUP35*^{ref}[*PSI*$^{+}$], but in the *SUP35*$^{\Delta N}$ segregants, the transmitted prion particles are quickly lost, resulting in Mendelian 2[*PSI*$^{+}$] : 2[*psi*$^{-}$] tetrads.[72]

Theoretically, in some [*PSI*$^{+}$] x [*psi*$^{-}$] crosses, interallelic complementation may occur. In this case, the conformational template of a weak [*PSI*$^{+}$] allele should interact with the DNA determinant of a [*psi*$^{-}$] allele providing strong [*PSI*$^{+}$] formation. As a result, the initial [*PSI*$^{+}$]/[*psi*$^{-}$] heterozygosity will cease, and the cell will become the [*PSI*$^{+}$]/[*PSI*$^{+}$] heterozygote which possesses two different [*PSI*$^{+}$] alleles with the same epigenetic mark but distinct in their protein sequences (see next section).

Interaction between different [*PSI*$^{+}$] alleles. When two distinct [*PSI*$^{+}$] alleles are combined in a diploid hybrid, their interaction may occur in several ways depending on the nature of difference. In the simplest case, both prion alleles are encoded by the same DNA determinant and differ just in their conformational templates, as in isogenic [*PSI*$^{+}$]^{S} x [*PSI*$^{+}$]^{W} diploids. All such diploids are phenotypically [*PSI*$^{+}$]^{S}, so the strong [*PSI*$^{+}$] variant is dominant over the weak one. Only dominant segregants are revealed in tetrad analysis allowing to suggest that [*PSI*$^{+}$]^{W} is less competitive in reproduction than [*PSI*$^{+}$]^{S} and is eventually lost.[50] However, Bateman and Wickner have demonstrated that at least in certain [*PSI*$^{+}$] strains different conformational templates coexist in "clouds" and can be separated from each other via cytoduction. Thus, the fate of [*PSI*$^{+}$]^{W} alleles in isogenic [*PSI*$^{+}$]^{S} x [*PSI*$^{+}$]^{W} hybrids is still questionable.[20]

If the combined [*PSI*$^{+}$] alleles differ in their DNA determinants but possess the same conformational template (like *SUP35*^{PNM2}[VH-1] and *SUP35*^{ref}[VH-1]), three types of the prion particles should be produced. Two of them correspond to the initial [*PSI*$^{+}$] alleles, and the third is mosaic; its amount depends on the efficiency of cross-templating. In tetrad analysis, the ascospores produced by such hybrids should contain a mixture of different [*PSI*$^{+}$] particles, but further, at the level of growing colonies, clear 2 : 2 ratio must be established, reflecting meiotic segregation of the DNA determinants. Here, the initial [*PSI*$^{+}$] alleles behave like classical Mendelian hereditary factors.

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In most complicated cases, when the combined [*PSI*$^{+}$] alleles differ from each other in both DNA and epigenetic determinants, various types of interaction are theoretically possible (Figure 3). They include (i) stable co-existence of the initial [*PSI*$^{+}$] alleles (Figure 3A), (ii) appearance of mosaic particles and "recombinant" [*PSI*$^{+}$] alleles in addition to the initial ones(Figure 3B), and (iii) competition between the initial and/or recombinant [*PSI*$^{+}$] alleles leading to eventual loss of the weakest one(s) (Figure 3C). Depending on the type of these interactions in certain diploid, the results of tetrad analysis may differ.

Combination-specific interplays between the DNA and epigenetic determinants in the [*PSI*$^{+}$] cells. Three types of such interplays are currently described. First, some combinations are lethal;[49,77] the mechanism of this phenomenon is still under discussion (for a review see ref. 79).

Second, in some combinations, the [*PSI*$^{+}$] particles are eventually lost, although the DNA determinant is quite appropriate for other [*PSI*$^{+}$] templates. The most famous example is elimination of certain *SUP35*^{ref}[*PSI*$^{+}$] alleles in the *SUP35*^{PNM2} background.[15,70,74,75,80,81] Interestingly, the "reciprocal" combinations are quite stable: overproduction of *SUP35*^{PNM2} induces the rise of specific *SUP35*^{PNM2}[*PSI*$^{+}$] alleles, templates of which are efficiently reproduced by *SUP35*^{ref}.[15]

In theory, combination-specific [*PSI*$^{+}$] loss may also occur due to positive selection of the [*psi*$^{-}$] state: if the [*PSI*$^{+}$] state is both unstable and lethal, only the [*psi*$^{-}$] derivates should survive, and the resulting cell culture will be totally cured. So, the second type of the interplays may be provided by different mechanisms.

Third, the interplay can lead to [*PSI*$^{+}$] template modification. For instance, the double substitution Q80K,Q81K significantly strengthens the template of *SUP35*^{ref}[*PSI*$^{+}$], and this effect is preserved even after prion transmission to the initial *SUP35*^{ref} background. The double substitution Q89K,Q90K gives an opposite effect (*sup35-M5* mutation). Notably, the conformational template of the resulting *sup35-M5*[*PSI*$^{+}$] allele fails to reproduce on the initial *SUP35*^{ref} background, thus manifesting the second type of the interplays.[70,82] So, transmission of a certain [*PSI*$^{+}$] allele from one DNA determinant to another and back is sometimes not a "true reversion".

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In the third type of the interplays, each combination of the DNA and the epigenetic determinants, when isolated in a single cell, may behave as a separate prion allele. However, in the first and the second types, the corresponding combinations *per se* are not prion alleles because of their inability to perpetuate in the progeny due to either lethal effect or [*PSI*$^{+}$] to [*psi*$^{-}$] conversion.

Non-multiplied or non-reproduced states of [*PSI*$^{+}$] alleles. Under the lack of Hsp104 chaperone function (for example, during GuHCl treatment or in strains with *HSP104* deletion), the [*PSI*$^{+}$] particles are not multiplied, and fail to produce new prion seeds.[39,40,83,84] As a result, the non-multiplied [*PSI*$^{+}$] particles (we propose to designate them by subscript, [*PSI*$^{+}$]$_{M-}$) are progressively diluted in cell divisions, and after approximately 15 cell cycles the overwhelming majority of the mitotic progeny is cured.[40,84,85] But the residual amyloid fibrils do not vanish: due to continuous Sup35p aggregation, they become extra long and usually remain in the mother cell because of asymmetric division in the *Saccharomyces* yeast.[86] However, the initial [*PSI*$^{+}$] allele appears to be intact, and may be multiplied and inherited after Hsp104 function is restored.[40,84,85] Thus, the [*PSI*$^{+}$]$_{M-}$ particles are hereditary factors, which retain their allelic specificity and can be potentially rescued for the progeny. We should also mention that [*PSI*$^{+}$] multiplication depends on the balance between various cellular chaperones, and any disturbance of this machinery may have remarkable consequences on the prion properties.[87–92]

In theory, the [*PSI*$^{+}$]$_{M-}$ state may exist even under normal chaperone function. This state could be characteristic to *SUP35* mutants in which the produced prion particles are not amenable for chaperone-mediated cleavage as a result of some defects in the Sup35p N-terminal region. However, such mutants are still unknown. And even if they do exist, the corresponding particles should be quickly cleared from the culture due to infinite enlargement and poor heritability.

Another atypical state of [*PSI*$^{+}$] alleles can be obtained when the prion particles are transmitted to a cell lacking both Hsp104 function and the DNA N-determinant. Under these conditions, when the [*PSI*$^{+}$] particles are neither reproduced nor multiplied ([*PSI*$^{+}$]$_{R-M-}$), the overwhelming majority of the mitotic progeny should be *SUP35*$^{\Delta N}$[*psi*$^{-}$], while some cells can retain a single or few prion particles.

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The [*PSI*$^{+}$]$_{R-M-}$ amyloid fibrils do not enlarge and thus are potentially heritable. They can be rescued through cytoduction to a *SUP35*^{ref} recipient with normal Hsp104 function.

The non-reproduced state of a [*PSI*$^{+}$] allele resembles "canonical" DNA allele in a non-replicative plasmid: both will be eventually lost in cell divisions, but might be rescued under specific conditions. This similarity gives additional support to applying the term "allele" for hereditary prions.

Implications of the bimodularity principle for other hereditary prions

Allelic diversity of amyloid hereditary prions other than [*PSI*$^{+}$] is less studied. However, the bimodularity principle is fully applicable to these prions also, as can be demonstrated by [*URE3*] and [*PIN*$^{+}$].

First, they depend on certain DNA determinants (*URE2* and *RNQ1*, respectively), and deletion of these genes lead to establishment of corresponding [*prion*$^{-}$] alleles (both deletions are not lethal).[34,93] Second, Ure2p and Rnq1p with reference protein sequence can form multiple [*URE3*] or [*PIN*$^{+}$] alleles differing in their phenotypic manifestation.[50,94–96] Third, alterations in the DNA determinant (point mutations or local deletions) may affect formation, stability, or phenotypic manifestation of prion allele, at least in [*PIN*$^{+}$].[97–100] Thus, [*URE3*] and [*PIN*$^{+}$] allele depend on both DNA and epigenetic determinants.

Non-amyloid hereditary prions (*C*, [*GAR*$^{+}$] and [β^{+}]) are also covered by the bimodularity principle. There are just three details to be mentioned. First, reproduction and multiplication of such prions are not separated from each other: it is the same molecular process. Second, as long as reproduction (multiplication) of non-amyloid hereditary prions is based on positive feedback loops without conformational templates (see above), their epigenetic determinants are of non-template nature. This does not impede the existence of different [*PRION*$^{+}$] epigenetic marks. For example, *C* requires all three components of the PaMpk1 pathway and is admittedly represented by the self-activating state of the whole cascade;[55] in that case, the DNA

determinant seems to be triple, *PaASK1—PaMKK1—PaMpk1*. Deletion or dysfunction of any gene involved in such DNA determinant should result in irreversible and inconvertible [*prion*$^{-}$] allele. [*GAR*$^{+}$] is provided by physical interaction between two non-homologous proteins Pma1 and Std1;[57] so, the corresponding DNA determinant is likely binary, *PMA1—STD1*. However, since [*GAR*$^{+}$] reproduces under *STD1* deletion,[57] the exact molecular basis of this prion is still questionable.

Conclusions

In this paper we have further developed the ideas of Chernoff and Tuite about prion alleles.[21,22] Prion allele is considered as a bimodular hereditary system which depends on a certain DNA sequence (DNA determinant) and a certain epigenetic mark (epigenetic determinant). The first encodes the prion protein sequence, while the second reflects the presence or absence of specific prion seeds. Bimodular designation of each prion allele (DNA determinant[epigenetic determinant]) is accordingly proposed.

It has been widely accepted that prions are "protein-only" hereditary factors. This is true *in vitro*, where native molecules of a certain prion protein are placed, and the only factor required for their prionization is addition of the corresponding prion seeds. But *in vivo* the situation differs markedly: when the DNA determinant is absent and native molecules are also lacking, there is no material for prionization even if the prion seeds are transferred to the cell. Thus, the "protein-only" concept is not universal and should be replaced by the bimodularity principle.

This principle is an appropriate generalization in prion studies, and its foresights can be found at least in several prion-related papers. For instance, amyloid prions are sometimes considered as conformational ("second order") templates in addition to DNA ("first order") ones.[4,101,102] This view is quite close to the bimodularity principle, but does not cover non-amyloid hereditary prions which reproduce via positive feedback loops without second order templates. The fact that prion function and evolution are affected at two levels (DNA and protein) has been recently pointed out by

462 Wickner and Kelly.[64] Notably, Bateman and Wickner denote the origin of different [*PSI*$^{+}$] alleles (A,
463 F and G) produced in the same *SUP35* background (E9) as [*PSI*$^{+}$E9A], [*PSI*$^{+}$E9F] and [*PSI*$^{+}$E9G].[20]
464 This approach is very similar to ours, but the DNA determinant is included within square brackets
465 and thereof looks like an element of the prion protein. However, when a certain [*PSI*$^{+}$] allele (for
466 example, [*PSI*$^{+}$E9A]) is transmitted to another *SUP35* background (Δ19 or ref), the resulting prion
467 alleles are designated as [*PSI*$^{+}$E9A]Δ19 and [*PSI*$^{+}$E9A]ref, where the initial DNA determinant is
468 written within square brackets and the new one is not. The bimodularity principle is devoid of the
469 aforementioned disadvantages. It gives useful and consistent designations of the DNA and
470 epigenetic determinants, prion alleles, their alterations, non-multiplied and non-reproduced states,
471 etc. (Table 2). Moreover, it is applicable to any hereditary prion allele, whenever it is amyloid or
472 non-amyloid.
473 In accordance with the bimodularity principle, we distinguish three types of prion allele differences.
474 They may affect the DNA determinant only, the epigenetic determinant only, or both. As a result,
475 multiple [*PRION*$^{+}$] and [*prion*$^{-}$] alleles can exist. Some of them are phenotypically distinct, while
476 others are similar in their manifestation, like DNA sequences with synonymous polymorphism.
477 Although prion alleles are considerably more complex hereditary factors compared to DNA alleles
478 and epialleles, there are a lot of remarkable similarities. Like “canonical” DNA alleles, prion alleles
479 are multiple and highly polymorphic. They can transiently exist in the non-reproduced state similar
480 to “canonical” DNA alleles expressed in a non-replicative plasmid. Alteration of a [*PRION*$^{+}$] allele
481 due to substitution of the DNA determinant is not a single-stage process resembling pre-mutational
482 DNA mismatch. Some prion alleles (for example, isogenic [*PSI*$^{+}$]^{S} and [*PSI*$^{+}$]^{W}) are dominant and
483 recessive, respectively, in the heterozygote. Moreover, in crosses like *SUP35*^{ref}[*PSI*$^{+}$] x
484 *SUP35*$^{\Delta N}$[*psi*$^{-}$], the combined prion alleles show clear Mendelian segregation.
485 Like epialleles, isogenic [*PRION*$^{+}$] and [*prion*$^{-}$] alleles differ from each other just epigenetically.
486 Moreover, being combined in a heterozygote, they are involved in paramutation establishment.
487 Thus, the term “prion allele” is appropriate for modern genetics.

It should be especially noted that the bimodularity principle is applicable not only for hereditary prion alleles, but for any epigenetic hereditary factor (Table 3). So, this is an important step towards universal genetic concepts which should embrace all variety of hereditary factors irrespective of their molecular nature.

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Figure legends

Figure 1. Main processes underlying heredity of amyloid prions. Mother cell and developing bud are separated by dashed line.

Figure 2. Molecular basics of [*GAR*$^+$] and *C*. A. The state of the PaMpk1 pathway in absence or presence of *C*. B. Preferable interactions of the Pma1 protein in the [*gar*$^-$] and [*GAR*$^+$] strains.[57]

Figure 3. Possible interactions between [*PSI*$^+$] alleles in heterozygote when both DNA and epigenetic determinants are distinct. A. No cross-seeding between different conformational templates occurs; only the initial prion alleles coexist. B. Cross-seeding between different conformational templates leads to prion allele recombination. The initial prion alleles coexist with two recombinant ones. C. Only one of recombinant prion alleles is stable.

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766 **Tables**

767 Table 1. Molecular mechanisms underlying formation and reproduction of hereditary prions

Mechanism	Prion	Protein determinant	Phenotypic effect	Organism	Refs.
Switch from native to amyloid conformation	[*URE3*]	Ure2	Alteration of nitrogen metabolism	*S. cerevisiae*	26
	[PSI^+]	Sup35	Nonsense suppression	*S. cerevisiae*	26,48
	[*Het-s*]	Het-s	Heterokaryon incompatibility in fuses with Het-S mycelium	*P. anserina*	103
	[PIN^+]	Rnq1	Induction of [PSI^+] *de novo* formation	*S. cerevisiae*	73
	[SWI^+]	Swi1	Alteration of carbon metabolism	*S. cerevisiae*	104
	[MOD^+]	Mod5	Drug resistance and cell survival under environmental stress	*S. cerevisiae*	38
Positive feedback through protein phosphorylation	*C*	PaMpk1 cascade	Crippled growth	*P. anserina*	53
Reproducible alterations in protein quaternary structure	[GAR^+]	Pma1 and Std1	Heritable switch in carbon source utilization	*S. cerevisiae*	57
Positive feedback through alteration in protein primary structure	[β^+]	PrB1	Constant activity of protease B	*S. cerevisiae*	59

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770 Table 2. Proposed abbreviations of prion alleles, their determinants, alterations and states on the example of prion [PSI^+]

Described parameter	Designation [(a)]	Genetic notion
DNA determinants	$SUP35^{ref}$	Reference *SUP35* sequence typical for laboratory strains, with natural polymorphism
	$SUP35^{alt}$	*SUP35* sequence differing from $SUP35^{ref}$ due to point mutations or local deletions (for example, $SUP35^{PNM2}$)
	$SUP35^{\Delta N}$	*SUP35* sequence lacking the N-domain coding region
Epigenetic determinants	[psi^-]	Absence of Sup35p amyloid state (prion null-allele)

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	$[PSI^+]$	Presence of Sup35p amyloid state
	$[PSI^+]^S$	Presence of conformational template corresponding to a strong prion variant
	$[PSI^+]^W$	Presence of conformational template corresponding to a weak prion variant
Prion alleles	$SUP35^{ref}[PSI^+]$	$[PSI^+]$ allele encoded by $SUP35^{ref}$
	$SUP35^{alt}[PSI^+]$	$[PSI^+]$ allele encoded by $SUP35^{alt}$
	$SUP35^{ref}[psi^-]$	Prion null-allele encoded by $SUP35^{ref}$
	$SUP35^{alt}[psi^-]$	Prion null-allele encoded by $SUP35^{alt}$
	$SUP35^{\Delta N}[psi^-]$	Prion null-allele encoded by $SUP35^{\Delta N}$
Alterations of prion alleles	$SUP35^{ref}[psi^- \rightarrow PSI^+]$	Alteration of a $SUP35^{ref}[psi^-]$ allele due to $[PSI^+]$ induction or epigenetic conversion
	$SUP35^{alt}[psi^- \rightarrow PSI^+]$	Alteration of a $SUP35^{alt}[psi^-]$ allele due to $[PSI^+]$ induction or epigenetic conversion
	$SUP35^{ref}[PSI^+ \rightarrow psi^-]$	Prion null-allele induction in a $SUP35^{ref}[PSI^+]$ cell via prion curing
	$SUP35^{alt}[PSI^+ \rightarrow psi^-]$	Prion null-allele induction in a $SUP35^{alt}[PSI^+]$ cell via prion curing
	$SUP35^{ref \rightarrow alt}[PSI^+]$	Alteration of a $[PSI^+]$ allele via replacement of $SUP35^{ref}$ with $SUP35^{alt}$ by transformation or cytoduction [(b)]
	$SUP35^{alt \rightarrow ref}[PSI^+]$	Alteration of a $[PSI^+]$ allele via replacement of $SUP35^{alt}$ with $SUP35^{ref}$ by transformation or cytoduction [(b)]
	$SUP35^{ref \rightarrow 1\text{-}123}[PSI^+]^{S \rightarrow U}$	Double alteration of the $SUP35^{ref}[PSI^+]^S$ or $SUP35^{ref}[PSI^+]^W$ allele after its transmission to the $SUP35^{1\text{-}123}$ background: replacement of the DNA determinant and dedifferentiation of the $[PSI^+]$ template
	$SUP35^{ref \rightarrow 1\text{-}123}[PSI^+]^{W \rightarrow U}$	
	$SUP35^{1\text{-}123 \rightarrow ref}[PSI^+]^{U \rightarrow S}$	Double alteration of the $SUP35^{1\text{-}123}[PSI^+]^U$ allele after its transmission to the $SUP35^{ref}$ background: replacement of the DNA determinant and spontaneous differentiation of the $[PSI^+]$ template
	$SUP35^{1\text{-}123 \rightarrow ref}[PSI^+]^{U \rightarrow W}$	
Prion allele states	$[PSI^+]_{R-}$	Non-reproduced state of a $[PSI^+]$ allele
	$[PSI^+]_{M-}$	Non-multiplied state of a $[PSI^+]$ allele
	$[PSI^+]_{R-M-}$	Non-reproduced and non-multiplied state of a $[PSI^+]$ allele
Homozygotes	$SUP35^{ref}[psi^-]/SUP35^{ref}[psi^-]$	Homozygote for prion null-allele encoded by $SUP35^{ref}$
	$SUP35^{alt}[psi^-]/SUP35^{alt}[psi^-]$	Homozygote for prion null-allele encoded by $SUP35^{alt}$
	$SUP35^{\Delta N}[psi^-]/SUP35^{\Delta N}[psi^-]$	Homozygote for prion null-allele encoded by $SUP35^{\Delta N}$
	$SUP35^{ref}[PSI^+]/SUP35^{ref}[PSI^+]$	Homozygote for a $[PSI^+]$ allele encoded by $SUP35^{ref}$
	$SUP35^{alt}[PSI^+]/SUP35^{alt}[PSI^+]$	Homozygote for a $[PSI^+]$ allele encoded by $SUP35^{alt}$
Heterozygotes [(c)]	$SUP35^{ref}[psi^-]/SUP35^{alt}[psi^-]$	Heterozygote for different $[psi^-]$ alleles
	$SUP35^{ref}[PSI^+]/SUP35^{\Delta N}[psi^-]$	Heterozygote for a $[PSI^+]$ allele and inconvertible $[psi^-]$ allele

	SUP35^{ref}[*PSI*$^{+}$]^{S}/*SUP35*^{ref}[*PSI*$^{+}$]^{W}	Heterozygote for two [*PSI*$^{+}$] alleles distinct in their epigenetic determinants (cloud heterozygote) [(d)]
	SUP35^{ref}[*PSI*$^{+}$]/*SUP35*^{alt}[*PSI*$^{+}$]	Heterozygote for two [*PSI*$^{+}$] alleles distinct in their DNA determinants [(e)]
	SUP35^{ref}[*PSI*$^{+}$]'/*SUP35*^{alt}[*PSI*$^{+}$]"	Heterozygote for two [*PSI*$^{+}$] alleles distinct in both DNA and epigenetic determinants; no cross-seeding (Fig. 3A)
Alterations of initial prion allele combinations	[*PSI*$^{+}$]/[*psi*$^{-}$→*PSI*$^{+}$]	Paramutation in initial [*PSI*$^{+}$]/[*psi*$^{-}$] heterozygote [(f)]
	[*PSI*$^{+}$]'/[*PSI*$^{+}$]"→'	Homozygotisation of the [*PSI*$^{+}$] conformational template in initial [*PSI*$^{+}$]'/[*PSI*$^{+}$]" heterozygote via epigenetic conversion [(f)]
	SUP35^{ref}[*PSI*$^{+}$]'/*SUP35*^{alt}[*PSI*$^{+}$]";rec	Cross-seeding between two [*PSI*$^{+}$] alleles distinct in both DNA and epigenetic determinants leads to their recombination. As a result, initial prion alleles coexist with two recombinant ones (fig. 3B)
	SUP35^{ref}[*PSI*$^{+}$]'/*SUP35*^{alt}[*PSI*$^{+}$]";rec^{ref}"	Only one of recombinant [*PSI*$^{+}$] alleles is stable (fig. 3C)
	SUP35^{PNM2}[*psi*$^{-}$]/*SUP35*^{ref}[*PSI*$^{+}$→*psi*$^{-}$]	Spontaneous [*PSI*$^{+}$] loss in initial [*psi*$^{-}$]/[*PSI*$^{+}$] heterozygote

[(a)] The simplest situations are considered. To describe more complicated ones, the proposed designations should be combined appropriately.

[(b)] Alteration of a [*PSI*$^{+}$] allele due to substitution of the DNA determinant is not a single-stage process. If the new DNA determinant is compatible with the initial conformational template, the prion fibrils become mosaic; they contain two Sup35p variants ("old" and "new"), and the portion of the former gradually grows up. This eventually results in complete loss of the "old" Sup35p variant, and from that moment the new [*PSI*$^{+}$] allele is established. Notably, the transitional stage between "old" and "new" prion alleles corresponds to classical pre-mutational DNA mismatch where the bases of both "old" and "new" alleles are simultaneously present. The main difference is that in case of prion alleles, the transitional stage is gradual and more prolonged.

[(c)] For each type of heterozygotes, only one certain example is shown. If a heterozygote is produced by genetic cross, the first written prion allele is of MATα parent. If a heterozygote is produced by genetic transformation of a haploid strain, the first written prion allele is encoded by chromosomal DNA determinant.

[(d)] Cloud heterozygocity may also occur in a single DNA determinant background (*SUP35*^{ref}[*PSI*$^{+}$]^{S,W}).

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782 [e] When two [*PSI*$^{+}$] alleles have the same epigenetic determinant, the corresponding Sup35p variants may co-aggregate producing a wide
783 spectrum of mosaic fibrils. This situation resembles the transitional stage between two prion alleles (see note 1), but here none of them is
784 eventually lost. The mosaic fibrils display the same hereditary features as a mix of the pure ones and therefore are not to be considered as
785 new prion alleles.

786 [f] Such alterations may occur on either *SUP35*^{ref} or *SUP35*^{alt} backgrounds.

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787 Table 3. Bimodularity of non-prionic epigenetic alleles

Mechanism	Organism	Certain allele	DNA determinant	Epigenetic determinant	Refs.
DNA methylation	*Arabidopsis thaliana*	*BAL* epimutation	*BAL* region of the chromosome 4	Hypomethylation of the *BAL* region	105
		Wild type		Normal methylation of the *BAL* region	
Histone modifications	*A. thaliana*	*FLC* silenced by vernalization	*FLC* region of the chromosome 5	H3K27me3 associated with the *FLC* region	106
		Wild type		Non-methylated H3K27 associated with the *FLC* region	
Positive feedback by means of transcription factors	*E. coli*	ON state of the bistable *lac* operon	The *lacY* and the *lacI* genes	Absence of the *lac* repressor	107
		OFF state of the bistable *lac* operon		Presence of the *lac* repressor	
Inhibition of translation in plastids by antibiotics	*Nicotiana tabacum*	Inherited *albino* phenocopy	Plastid genes for ribosomal proteins	Absence of plastid ribosomes	108
		Wild type		Presence of plastid ribosomes	
Reproducible differences in cortex structure	*Paramecium sp.*	Inverted ciliary rows	The genes encoding cortical proteins	Inverted position of ciliary basal bodies	109
		Wild type		Wild type position of ciliary basal bodies	

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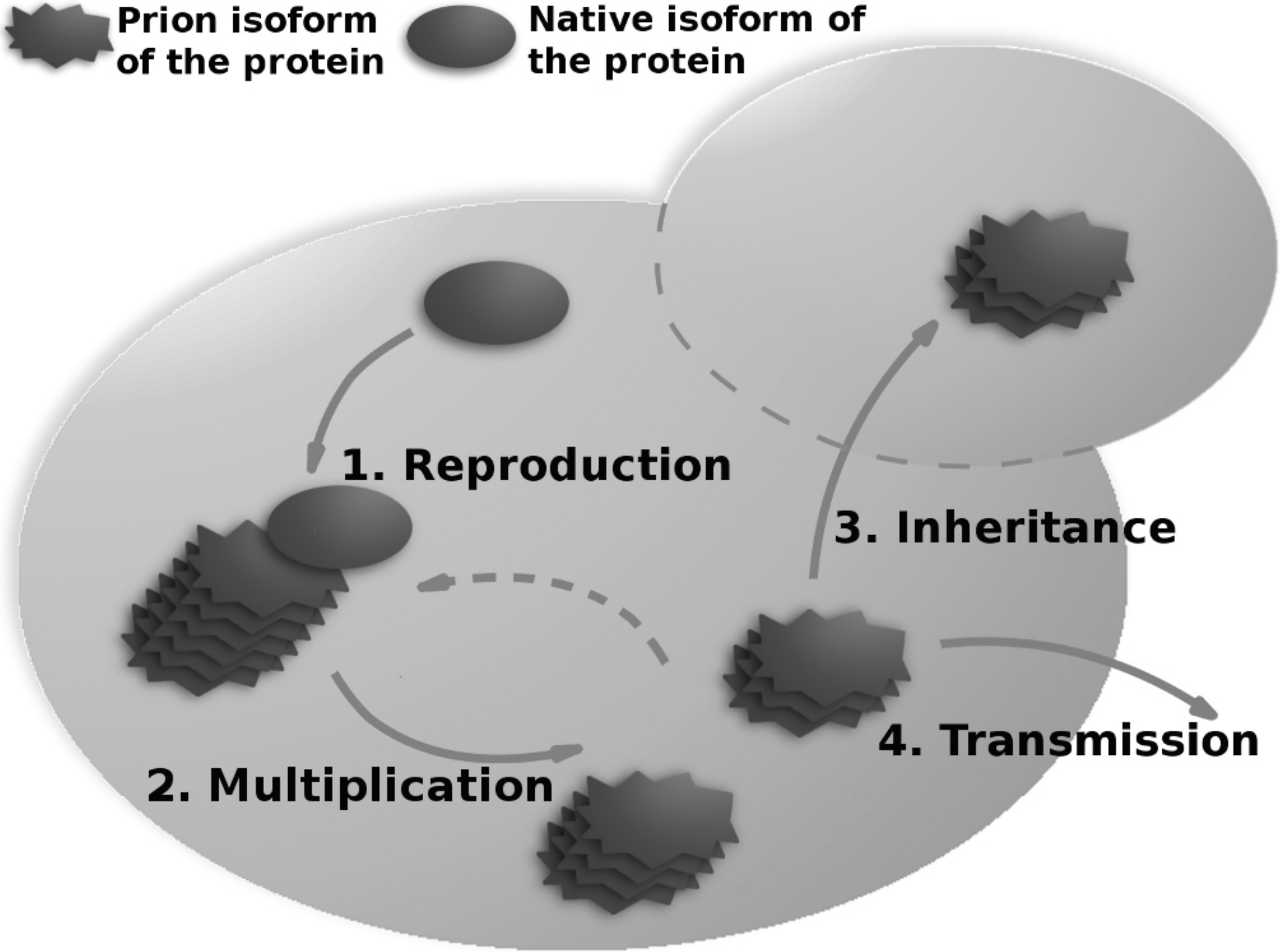
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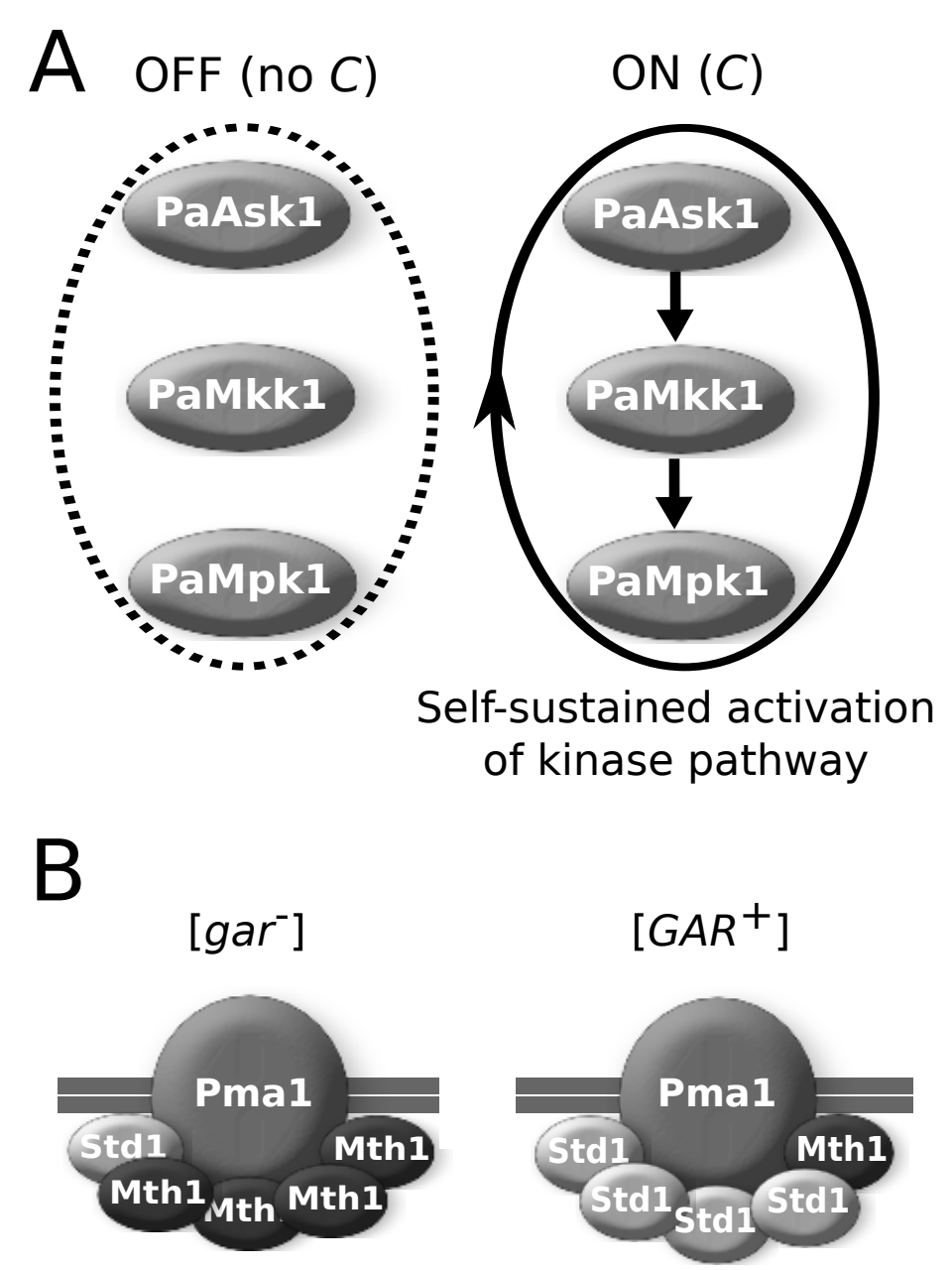
Native isoform of the protein
1. Reproduction
2. Multiplication
3. Inheritance
4. Transmission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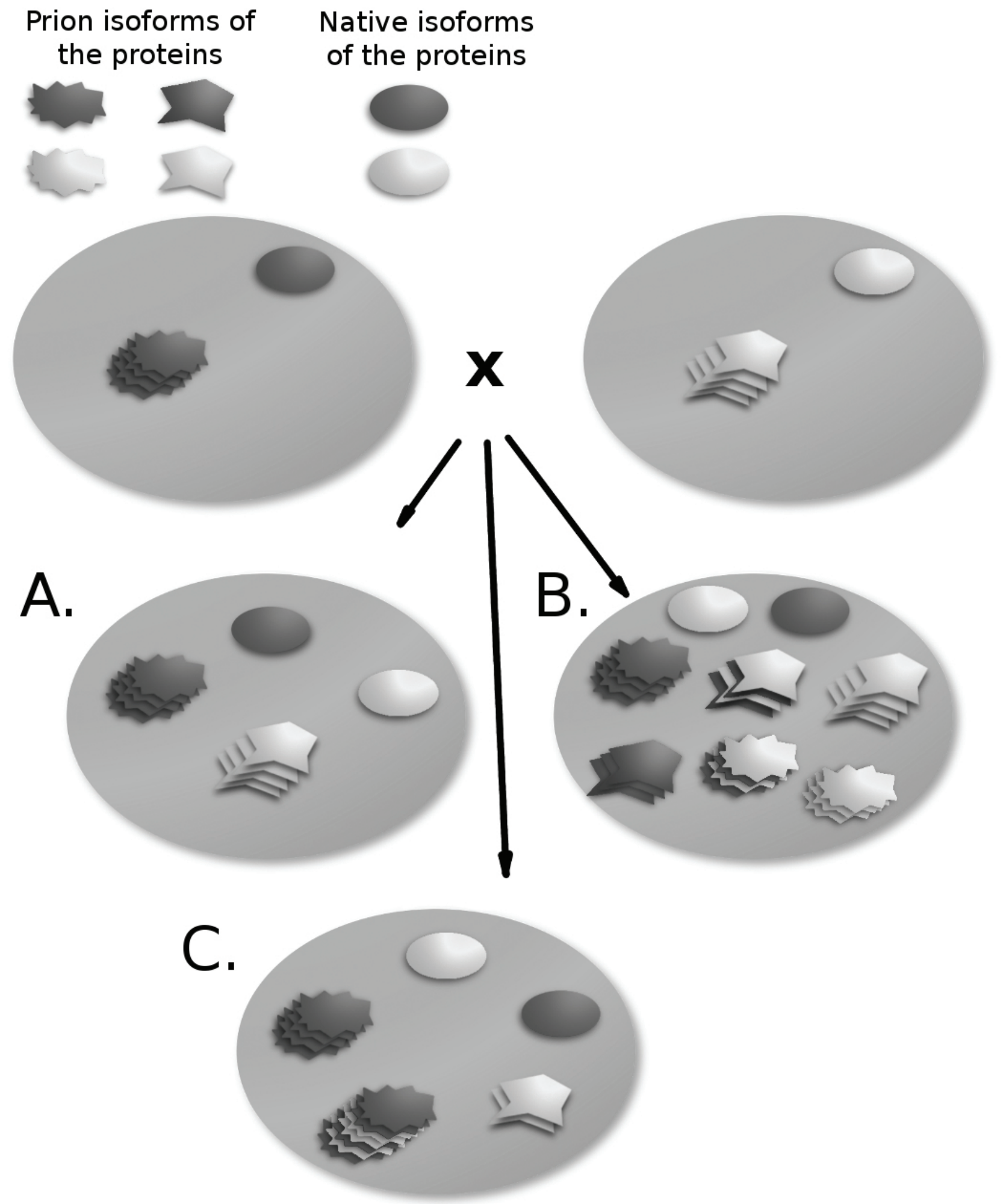
Prion isoforms of the proteins
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